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THE INHIBITION OF SEEDLING GROWTH BY CROP RESIDUES IN SOIL INOCULATED WITH *PENICILLIUM URTICAE* BAINER *

by D. E. BEHMER and T. M. McCALLA **

INTRODUCTION

In the Great Plains area of the United States, stubble-mulch practices have been widely used in the control of soil erosion by wind or water. Although this method of leaving crop residue on the surface has been very effective in combating erosion, depressed plant growth and reduced grain yield have often occurred in wet years. Similar growth problems have been observed during unseasonably cool, wet springs in field experiments at Lincoln, Nebraska. Because increased numbers of certain micro-organisms occur immediately beneath a decomposing surface mulch, the production of phytotoxic compounds under these conditions may well have been associated with the adverse effect on plant growth. In fact, a strain of *Penicillium urticae* Bainer isolated from stubble-mulch plots at Alliance, Nebraska, has been found to produce a phytotoxic substance when grown in culture media (Norstadt and McCalla [6]).

Effects of microbial decomposition of plant residues on seedling growth have been studied by numerous investigators. Patrick [7] showed that fumigation of a peach-orchard soil prevented the development

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of a substance toxic to peach tree seedlings. Swaby [8] tested plant response to incorporations of organic matter in sterile and inoculated sand. He concluded that the addition of organic matter under sterile conditions does not influence growth, but that the microbial decomposition of organic matter may produce plant-growth-inhibitory substances.

Culture filtrates of *Aspergillus niger* will induce severe curvature of corn-seedling roots (Curtis [2]). The growth substance in the culture filtrate has been isolated and identified as a neutral cyclic peptide compound that has been given the name *malformin*. A filtrate that induced curling of roots and caused reduced root and shoot growth of wheat seedlings was produced by a culture of *P. urticae* (Norstadt and McCalla [6]). One of the toxic substances from the culture filtrate was identified as *Patulin*. Several workers have shown that crop residue extracts depress plant growth (Collison and Conn [1], Newton and Young [4], Nielsen, Cuddy, and Woods [5]). Aqueous extracts of sorghum, corn, oats, sweetclover, wheat, soybean, and brome-grass residues were toxic to corn, wheat, and sorghum seedlings (Guenzi and McCalla [3]).

The purpose of this study was to determine if wheat-seedling growth inhibitors are present in crop residues in the soil, and if *P. urticae* produces substances toxic to wheat seedlings in residue-treated soil.

MATERIALS AND METHODS

Samples of the plow layer of a Sharpsburg silty clay loam soil were taken from the Agronomy Farm, Lincoln, Nebraska, on an east slope of 4 per cent. The dark-colored, friable surface layer of 8 to 12 inches covers a finer textured zone which grades into a light-brown parent material of Peorian loess origin. The soil samples were composited, air dried, and passed through a 2 mm sieve. The bulk soil was divided into four parts, three of which were separately mixed with 1% by weight of finely ground (to pass 40-mesh screen) alfalfa, corn and sorghum stover residue. No residue was added to the fourth part. From each of the four parts of soil or soil with residue, eighteen 50-g samples were weighed into individual 100-mm petri dishes, and the dishes and contents were autoclaved for 2 hours. Nine dishes of each type were then inoculated with a suspension of *P. urticae* spores in modified Crone's solution (7.1 g NH_4NO_3 and 3.6 g K_2HPO_4 per liter of distilled water). Spores for inoculation were harvested from 8-week-old rose-bengal slant cultures. The count of viable spores in the inoculating suspension was approximately 15,000 per ml. A sufficient volume of suspension was used to bring the moisture content of the soil to 40% (1 moisture equivalent equals 27% moisture). To the remaining nine petri dishes of each type, sterile Crone's solution was added in sufficient quantity to bring the moisture level of the soil to 40%. The experiment thus included 8 treatment combinations of residue and inoculum, with each treatment represented by 9 petri dishes.

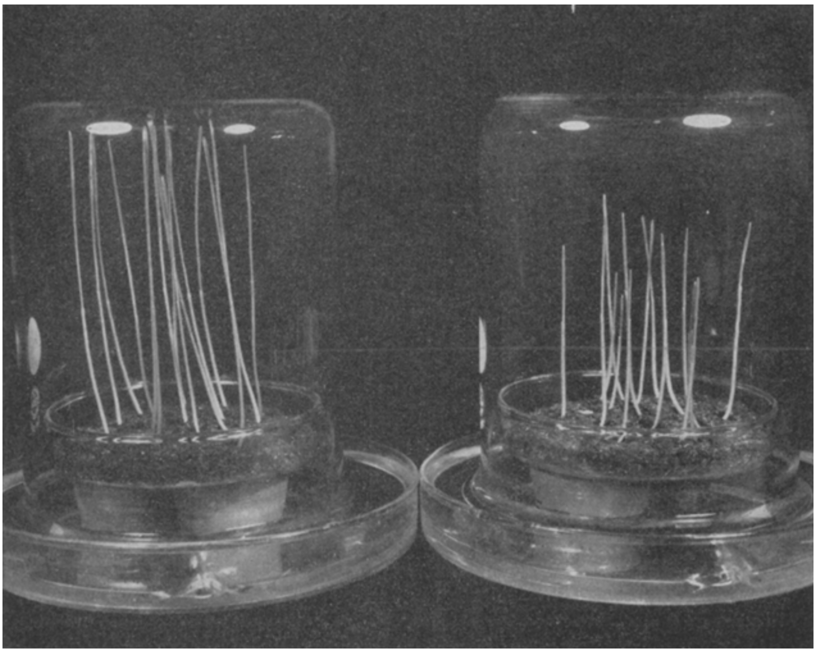

Fig. 1. Humidity chambers used in the experiment. Component parts are: 1 large petri plate, 2 large rubber stoppers, and a 1-liter beaker.

All 72 petri dishes were incubated at 24°C in a moisture-saturated atmosphere. After 2 weeks of incubation, 3 dishes of each treatment were planted with 15 fungicide-treated * Cheyenne winter-wheat seeds. Wheat seeds were planted in a second 3 dishes of each treatment after 3 weeks of incubation, and in the final 3 dishes after 4 weeks of incubation. Planted plates, without lids, were placed in humidity chambers, as shown in Figure 1. Data on seed germination and on length and weight (oven-dry basis) of the seedlings were recorded after 7 days of growth at 24°C.

* Orthocide – active ingredient, N-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide.

EXPERIMENTAL RESULTS AND DISCUSSION

The influence of crop residues, *P. urticae* inoculation, and different incubation periods (prior to planting) on the germination and growth of wheat seedlings in soil is shown in Table 1, and an analysis of variance is shown in Table 2.

TABLE 1

Weight * (oven-dry basis) and length * of wheat shoots, after 7 days of growth, and germination percentage * of wheat in *P. urticae*-inoculated and non-inoculated soil containing 1% by weight of finely ground alfalfa, corn, and sorghum residues.

Residue treatment	Soil incubation period **						Mean
	2 weeks		3 weeks		4 weeks		
	Non-inoc.	Inoc.	Non-inoc.	Inoc.	Non-inoc.	Inoc.	
Weight of wheat shoots (mg)							
None	99	110	103	107	93	96	101
Corn	104	102	98	104	86	98	99
Sorghum	94	101	93	96	93	86	94
Alfalfa	86	91	86	80	70	62	79
Mean	96	101	95	97	86	86	
Length of wheat shoots (mm)							
None	132	133	128	125	114	127	126
Corn	117	99	118	101	100	103	106
Sorghum	111	89	100	86	103	91	97
Alfalfa	93	76	88	62	67	58	74
Mean	113	99	108	94	96	95	
Germination (%)							
None	93	100	98	100	100	98	98
Corn	89	100	93	100	98	80	93
Sorghum	100	100	98	100	100	98	99
Alfalfa	98	100	98	100	98	89	97
Mean	95	100	97	100	99	91	

* Mean of three replications with a total of 45 seeds.
** Time elapsed from wetting to planting, incubation at 24°C.

Addition of corn and sorghum stover and alfalfa hay residues to the soil effectively reduced yields as compared to the soil without residues. Alfalfa residues reduced both length and weight of shoots to the greatest extent.

Seedling shoot-length was reduced in all cases by addition of

TABLE 2

Analysis of variance of length and weight (oven-dry basis) of wheat shoots, after 7 days of growth, and germination percentage of wheat in *P. urticae*-inoculated and non-inoculated soil containing 1% by weight of finely ground alfalfa, corn, and sorghum residues.

Source of variation	Degrees of freedom	Mean square		
		Wheat shoots		Germination
		Length	Weight	
Residues	3	8,561.8**	1,627.8**	1.22
Incubation periods	2	710.3**	1,702.8**	2.39
Residues × incubation periods	6	132.9	112.4	1.22
Inoculation	1	1,859.5**	6.3	0.50
Inoculation × residues	3	398.9**	82.3	0.76
Inoculation × incubation periods	2	351.3**	174.4	4.50*
Inoculation × residues × incubation periods	6	40.0	36.8	0.93
Error	48	74.2	98.6	1.29

* Significant at the 5% level
** Significant at the 1% level

residue to the soil. Furthermore, in most cases inoculation with *P. urticae* caused an additional stunting of the shoots (Fig. 2).

Increasing the length of the incubation period prior to planting caused a slight reduction in seedling weight and length on the control soil and the corn and sorghum-treated soils. Soil treated with alfalfa residues became progressively more toxic to wheat-seedling growth with length of incubation. The high toxicity of incorporated alfalfa residue suggests the presence of natural plant-growth inhibitors. However, inoculation of the alfalfa residue with *P. urticae* increased this toxic effect, especially with increased incubation time prior to planting.

Wheat seedlings grown in soil treated with alfalfa residue, inoculated with *P. urticae,* and incubated for 3 and 4 weeks prior to planting, developed a root curling not evident with other residue treatments (Fig. 3). There was also some indication of root curling at the 2-week incubation period.

Although differences were not statistically significant, germination of wheat appeared to be quantitatively improved in soil inoculated with *P. urticae,* except at the 4-week incubation period. The interaction between inoculation and incubation period was shown to be significant at the 5% level of probability.

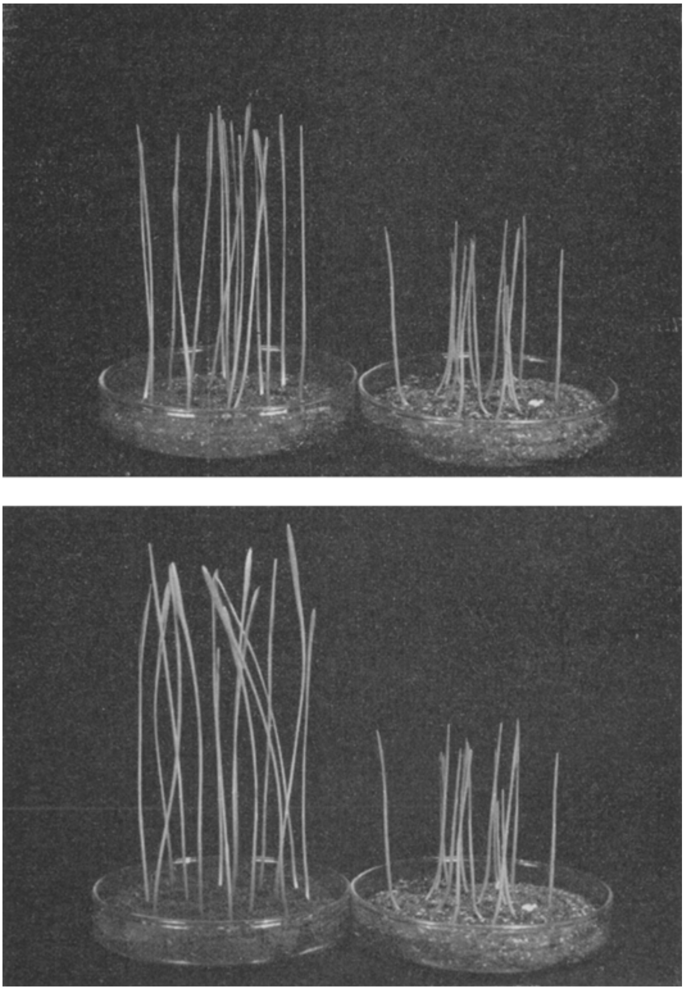

Fig. 2. Appearance of 7-day-old wheat seedlings growing on Sharpsburg silty clay loam soil incubated 3 weeks prior to planting.

Upper, containing 1% incorporated alfalfa residue – *left*, non-inoculated; *right*, *P. urticae* inoculated.

Lower, containing (*left*) no residue; and (*right*) 1% alfalfa residue. Both plates received *P. urticae* inoculation.

Any effects of adverse C/N ratio caused by the addition of residue to this soil should have been offset by the nitrogen present in the wetting solution (Crone's medium). Autoclaved *P. urticae*

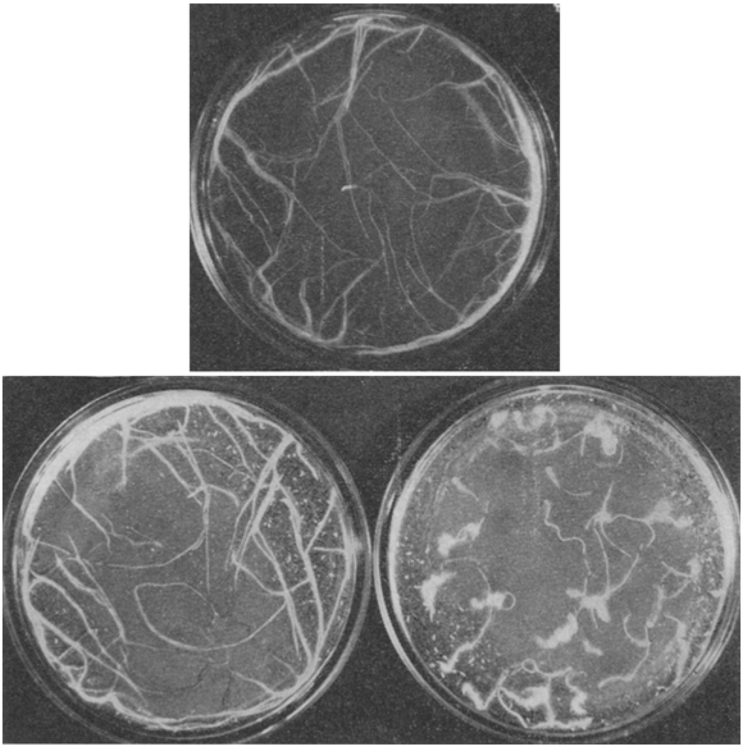

Fig. 3. Root patterns of 7-day-old wheat seedlings growing in moist Sharpsburg silty clay loam soil. Soil was incubated for 4 weeks prior to planting of the wheat.

Upper, containing no residue; *lower, left*, 1% incorporation of alfalfa residue, non-inoculated; *lower right*, 1% incorporation of alfalfa residue, *P. urticae*-inoculated.

might have been added to the non-inoculated soil as a proof that growth effects were attributable to the viable mold. However, at this very low concentration it is doubtful that there was any effect, since the inoculated control soil outyielded the non-inoculated control soil throughout the experiment.

Although residues alone effected a reduction in seedling growth, *P. urticae* caused reduced growth only in the presence of residues. These findings suggest that crop residues in soils, alone or in combination with *P. urticae*, may cause depressive effects on the growth of wheat seedlings. The presence of certain soil-inhabiting fungi and their action on crop residues may induce growth retardation in plants whose root systems are exposed to degradation products of crop residues. However, these relationships have not been demonstrated in the field.

SUMMARY

Laboratory experiments were conducted to determine the effects of crop residues, without and with *Penicillium urticae* Bainer inoculation, on growth of wheat seedlings in soil. Fifty grams of Sharpsburg silty clay loam soil, containing 1% by weight of incorporated alfalfa, sorghum and corn stover residue, were placed in petri dishes, autoclaved, wetted to 40% moisture, and incubated at 24°C. for periods of 2, 3, and 4 weeks. One-half of the petri dishes were inoculated with *P. urticae*. Germination and seedling-shoot measurements were taken after 7 days of growth.

The results of this study showed that (1) inoculation of soil generally reduced seedling height regardless of the residue treatment; (2) inoculation of soil containing corn and sorghum residues resulted in greater tissue production but reduced height of seedlings as compared to non-inoculated soils; and (3) in the absence of residues, the inoculated control soils were a better growth medium for wheat seedlings than were the non-inoculated control soils. In addition, alfalfa residues, especially in the presence of *P. urticae*, were strongly inhibitory to the wheat seedlings, causing curling and reduced wheat-seedling root growth.

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